IHC meeting, International Symposium on Authenticity and Quality of Bee Products and 2nd World Symposium on Honeydew Honey, Chania, Greece, 7-10 April, 2010

Abstracts

CRETAN BEEKEEPING IN THE PAST

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The products of bees have been used in the island of Crete from deepest antiquity and according to myth, when Zeus was born, his mother Rhea determined to save him from his father's clutches. The infant Zeus was concealed in a cave on Mount Dicte, which was full of sacred bees, who fed him on honey, and the goat Amaltheia gave him her milk. The cave was guarded by armed men, the Kuretes, who, when Zeus cried, clashed their armour and made a great noise so that his father Kronus should not hear him. Here in Crete the young god was reared, and when he grew up, after waging a ten-years war against his father, defeated him, and himself became King of the gods who lived in Olympus.

Another myth says that no one, god or man, might enter this cave where Zeus was hidden, and from which at certain seasons a light streamed forth. Four Cretans, however, attracted by the honey, which they meant to steal, protected themselves with armour and managed to enter the cave. As they began to take the honey, they saw the cradle and swaddling clothes of Zeus, and, at the sight, the joints of their armour burst and it fell off. Thus unprotected they were attacked by the sacred bees.

There are many variants of the birth myth, but one feature appears in all: the infant Zeus was fed either by bees or by nymphs, who fed him on honey.

In the old myth of Glaucus, the son of Minos and Pasiphae, the honey played the role of mediator between life and death. Glaucus fell into a jar of honey and drowned. The seer Polyidus found, preserved in the honey, the body of the boy and restored life to him with a certain herb.

In the tablets of Linear B – the first Greek writing system that was used between the 14th and 12th century B.C. – the honey occurs with the same word (me-ri = meli) that is in use today, 3,500 years later, in Modern Greek. The wax was known as ke-ro, the honey comb as ke-ro + ideogram *172, and probably the beekeeper as me-ri-te-wo (melitēwos - melisseus).

In Minoan Mycenaean world, as well as in classical times, honey was offered to the gods. Some tablets of Knossos inform us of the offering of a jar of honey to Poseidon, to Eleuthia (Eileithyia), to the "all gods" and to the Mistress of the Labyrinth.

At the settlement of Mochlos, on the north coast of east Crete, the archaeologists discovered, among other findings, ceramic lamps and conical cups, which were used as lamps during the Late Minoan I period (1600-1450 BC). The examination of residues found in them identifies beeswax and the researchers consider that beeswax was used as fuel in lamps. According to one opinion, beeswax could not have been used as a lamp fuel unless it was a fairly common material. This means there must have been a fairly extensive beekeeping industry in Crete in this period.

In some places in Crete pottery vessels were discovered, dated between 1750-1450 BC, which look like traditional pottery smokers that were used in Greece until a few decades ago. A copy of one of these vessels that was found in Zagros was used lately experimentally as a smoker in beekeeping. The experiment confirmed that these vessels can be used successfully as bee smokers.

However, beehives from the Minoan period have not yet been discovered. Several researchers believe that some pottery vessels, in the shape of a cylinder tapering downward with a hole in its bottom center, were used as beehives. But chemical examination of residues in these vessels, which could confirm their use as hives, has not yet offered proof. At the moment, the oldest certain examples of beehives excavated in Crete are dated to the 2nd century BC. It is difficult to explain why beehive forms do not reach Crete for several hundred years after they are widespread across the Greek mainland. Perhaps this fact means that the Cretan beekeepers of this period used hives made from perishable materials (wicker, wood, etc).

The honey of Crete was famous in antiquity. Several ancient authors, such as Pliny the Elder (1st c. AD) and Dioscourides (1st c. AD) mention the excellence of Cretan honey and wax, and although they do not specify the towns that produced it. Only Diofanes (1st c. BC, quoted from Kassianos Vassos – 6th c. AD) inform us that the best Cretan honey was this from Akramanmorion. The honey of Crete is also mentioned in Greek papyri in Egypt, where it was imported because of its quality.

Examples of horizontal clay beehives from the Byzantine period were also excavated in Crete, but we do not know much about Cretan beekeeping in this period. However, the very interesting traditional beekeeping in Crete, helps us to understand the beekeeping in previous periods.

The beekeepers in the eastern part of the island used conical horizontal terracotta hives, while their colleagues from the central and western parts used hives with movable combs (top-bar hives). Abbot Della Rocca from Syros was the first who wrote, in 1790, about the top-bar hives used in Crete. These hives was cylindrical, lay vertically - of course - and were made from clay or wicker.

It is not very well known but the beekeepers from Crete used in some cases, apart from cylindrical top-bar hives, rectangular ones, made of wooden boards. These hives was with sloping sides and equal bars, like the “Kenya Top-Bar Hive”. In other words, the Cretan beekeepers used hives identical to the “Kenya Top Bar Hive”, before it was discovered and recommended as the best solution for beekeeping in Africa and the developing world.

Traditional hives, of all used types, were still in use in Crete until about twenty years ago. In present days a small number of beekeepers, mainly to keep traditions alive, use, in addition to modern hives, some "vraski" hives (clay top-bar hives).

ENTOMOLOGICAL ORIGIN OF STINGLESS BEE HONEY, AS A CONTRIBUTION OF PROF. JOÃO MARIA FRANCO DE CAMARGO, AN EXPERT IN BIOGEOGRAPHY

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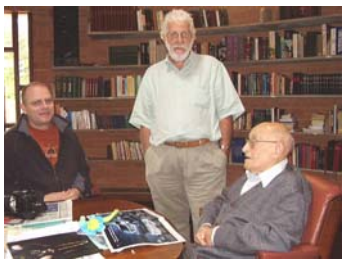
Our grateful memory to Prof. JMF Camargo deserves a special time in the ancient island of Crete, with the Greek hospitality "xenia" and the elegant beauty of the Maich auditorium. The scientist and illustrator of Meliponini. A discrete man who was born in Anhembi 20.06.41 and passed away in Ribeirão Preto 07.09.09, São Paulo, Brazil.

His interest for the stingless bees (Meliponini) was initiated during his first expedition to the Amazone during the year 1963, an awakening for his unique awe caused by the work of these bees. With the innocence of his 22 years, he started a collection with unpredictable growth and size, with the unconditional support of Prof. W Kerr. Today the collection has a 250.000 bee specimens, pin-mounted and liquid-preserved, 150.000 of them are Neotropical Meliponini. In the last years, this collection reached a considerable informative level. Ambitious projects on taxonomy, and especially on historical biogeography, were tackled to postulate hypothesis on patterns of vicariance/cladogenesis of great significance for the comprehension of evolution and the origin of the biodiversity of the Neotropical fauna. More than 16.000 km of river ways to study bees in their forest environment. His contribution reached 90 new taxa, 3 genera and 87 species that he named after native indians, locations, colleagues, friends and his wife.

The Venezuelan stingless bees were identified by Prof. Camargo for the entomological origin of honey: *Frieseomelitta paupera, Frieseomelitta* sp. group *varia, Melipona compressipes, Melipona crinita, Melipona eburnea, Melipona favosa, Melipona fuscopilosa, Melipona lateralis, Melipona paraensis, Melipona trinitatis, Melipona* sp., *Melipona* sp. group *fulva, Nannotrigona* sp. *aff. Chapadana*, *Plebeia* sp., *Scaura* aff. *Latitarsis, Scaptotrigona* sp. 1, *Scaptotrigona* sp. 2, *Scaptotrigona* sp. aff. *Polystica, Tetragona clavipes, Tetragonisca angustula*. Many other species were also identified to pin point locations where they grow.


Before his conference in Venezuela observing a nest in the wall, Barinas (March 2008). Three generations of Meliponini entomologists (C Rasmussen, JMF Camargo, his tutor Father JS Moure), Batatais. Prof. Camargo with the RPSP collection of bees, Ribeirão Preto (April 2008).

BOOK PROJECT "POT HONEY: A LEGACY OF STINGLESS BEES"

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Stingless bees (Meliponini) produce honey in pots since the late Cretaceous, and up to now (2010) the Codex Alimentarius Commission has not included this product in the honey regulations. These bees are well known in tropical and subtropical regions, but they produce less honey than *Apis mellifera*. Their honey is stored in pots made up with cerumen, not in beeswax combs. A group of scientist working on pot honey have agreed to join efforts to provide information on several topics of a honey received as a legacy of a great biodiversity of stingless bees in the world, almost 400 group species from the Neotropics. It is a great pleasure to inform the content of the book in our annual of the International Honey Commission in the island of Crete, and hope more contributions will join this effort. Tha last conference given by Prof. Camargo in Venezuela (2008) was kindly translated by Dr. Roubik DW and reviewed by Dr. Pedro SRM: 1. Historical biogeography of the Meliponini (Hymenoptera, Apidae, Apinae) of the Neotropical region. Tentative titles are given following the alphabetical order of authors: 2. Palynology serving the stingless bees (Barth OM, Brazil). 3. Physicochemical composition of stingless bee *Tetragonisca angustula honey* in comparison with *Apis mellifera* honey (Almeida-Muradian LB and Leal Souza G, Brazil). 4. Analytical treatment of bee products for the determination of bioelements and toxic products (Di Bernardo ML, Venezuela). 5. The Mayans and the melliferous flora of Yucatan: Xtabentum (*Turbina corimbosa*), Tah (*Viguiera dentata*), Tzitzilche (*Gymnopodium floribundum*) and Cucurbitaceae (Echazarreta C, and García Quintanilla A, Mexico). 6. Volatile and semivolatile compounds of honeys (Fernández-Muiño MA, Spain). 7. Antibacterial activity of stingless bee products (Gil F, Venezuela). 8. Microbiological ecology of a *Tetragonisca angustula* hive (González AC, Venezuela). 9. Traditional vs rational stingless bee beekeeping (Pereira FM and Lopes MTR, Brazil). 10. Non-aromatic organic acids of honeys (Sancho-Ortiz MT, Spain). 11. Impact of fragmentation on Meliponini (Soares AEE, Brazil). 12. Cultural aspects of meliponiculture in the Americas (Souza BA). 13. New approach to the antioxidant capacity (Rodríguez-Malaver AJ, Venezuela). 14. Sensory appreciation of pot honey (Vit P, Venezuela). Not sure who will write on marketing but possibly from Lune de Miel, 15. Marketing prospects of an ancient honey (Morlot M, France). In the appendix we will have the Harmonized Methods of International Honey Commission (IHC), by Bogdanov S.

This book will collect a state of the art for an infant industry, as Dr. Heard TA named meliponiculture in Australia. We hope that a worldwide distribution with the Taylor & Francis Group will interest the readers of our comprehensive coverage focused to the knowledge and value of a honey that we agreed to name pot honey.

A STINGLESS BEE DATABASE ON A WEBSITE REPOSITORY

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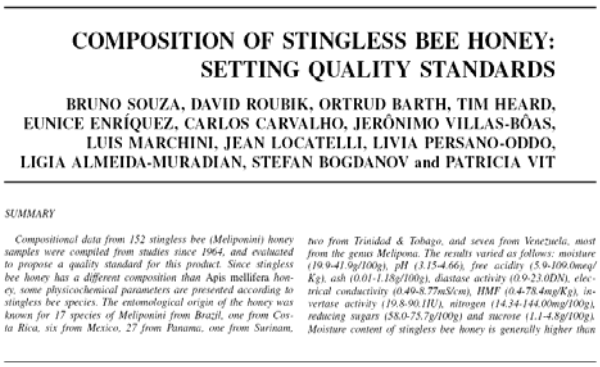
A repository consists on a great store of data with free access, availability of data characteristics of origin and quality, data of authors and responsible staff to capture data, projects from where they derived, institutional affiliations and collaborators.

Meliponini lived longer than Apini, their oldest fossil dates back to the Superior Cretaceus. The genus *Apis* has 11 species, while Meliponini have a 60 genera. Therefore the greater biodeversity of Meliponini generates a wider variety of pot honey types, if compared with *Apis* comb honeys.
After the laborious dossier produced to write the Interciencia 2006, 31(12): 867-875 article, I understood that it was necessary to compile and facilitate retrieval of analytical data of stingless bee honey, for comprehensive understanding and applications in future.



COMPOSITION OF STINGLESS BEE HONEY: SETTING QUALITY STANDARDS

BRUNO SOUZA, DAVID ROUBIK, ORTRUD BARTH, TIM HEARD, EUNICE ENRÍQUEZ, CARLOS CARVALHO, JERÔNIMO VILLAS-BÔAS, LUIS MARCHINI, JEAN LOCATELLI, LIVIA PERSANO-ODDO, LIGIA ALMEIDA-MURADIAN, STEFAN BOGDANOV and PATRICIA VIT

SUMMARY

Compositional data from 152 stingless bee (Meliponini) honey samples were compiled from studies since 1964, and evaluated to propose a quality standard for this product. Since stingless bee honey has a different composition than Apis mellifera *honey, some physicochemical parameters are presented according to stingless bee species. The entomological origin of the honey was known for 17 species of Meliponini from Brazil, one from Costa Rica, six from Mexico, 27 from Panama, one from Suriname, two from Trinidad & Tobago, and seven from Venezuela, most from the genus Melipona. The results varied as follows: moisture (19.9-41.9g/100g), pH (3.15-4.66), free acidity (5.9-109.0meq/Kg), ash (0.01-1.18g/100g), diastase activity (0.9-23.0DN), electrical conductivity (0.49-8.77mS/cm), HMF (0.4-78.4mg/Kg), invertase activity (19.8-90.1IU), nitrogen (14.34-144.00mg/100g), reducing sugars (58.0-75.7g/100g) and sucrose (1.1-4.8g/100g). Moisture content of stingless bee honey is generally higher than*

The difficulty to obtain and to share bibliographical references on compositional studies of Meliponini honeys, generated the idea to organize the information available in a web page. The database shows the entomological and geographical origin of the honeys with their profiles of physicochemical composition, melissopalinology, bioactivity, geographical origin, bee management, methods of extraction, processing and storage, analythical methods and references. The entomologists Camargo JMF, Pedro SRM and Ayala R are visible in one section. Also the table odour-aroma used for stingless bee honey. Contributions are received from all over the world. The analysts of stingless bee honeys can send their contributions in the format guideline. It is suggested to send the original document (report, thesis, etc.) or the portions with the info, to backup the authenticity of data.
The stages to build up the website http://saber.ula.ve/stinglessbeehoney consisted in the following steps: 1. Capture of requirements. 2. Compilation of the information. 3. Architectural design of the site. 4. Graphic design of the site. 5. Expression in XHTML and CSS. 6. Proofs and corrections. 7. Publication of the site in the web server. 8. Manteinance and periodical backup of the information.
Our current collection has eight entries for a total of 106 honeys (104 from stingless bees and 2 from *Apis mellifera*) 001 Venezuela 01 (43), 002 Venezuela 02 (27), 003 Argentina 01 Australia 01 Brazil 01 Venezuela 03 (6), 004 Venezuela 04 Guatemala 01 (2), 005 Portugal 01 (1 + 1 *Apis*), 006 Venezuela 05 (2), 007 Peru 01 (15 + 1 *Apis*), 008 Australia 02 (8). These bees are illustrated in galleries of their countries of origin Argentina, Australia, Brasil, Guatemala, Peru and Venezuela.

Links of the page are provided for: 1. Meliponini: Catalogue of Neotropical Meliponini, Camargo & Pedro, Literature and Webbee. 2. Bee journals: Apidologie, Bees for Development and Apiacta. 3. Sites of interest. APIMONDIA and Bee hexagon.
The database Stingless Bee Honey, should become in the near future a data repository with all the meta-information and retrieving facilities available, in agreement with the open-access academic philosophy.

Acknowledgements are given to Rodrigo Torrens and Lady Rivera from Centro de Teleinformación, Parque Tecnológico de Mérida, Universidad de Los Andes, Mérida, Venezuela.

IMPROVEMENTS OF PHADEBAS® FOR THE PHADEBAS® HONEY DIASTASE TEST

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The Phadebas method is one of two methods recommended by the International Honey Commission for determination of Diastase activity in honey. The Phadebas Amylase test, used for the method is manufactured and sold by Magle Life Sciences. The Diastase activity given by this method is based on fixed equations determined through a study comparing the Schade method with one specific batch of Phadebas tablets[1]. However, since there may be differences in reactivity between Phadebas batches, using predetermined equations will lead to varying results. For other Phadebas applications this is not a problem as quantification is based on the batch specific standard curve supplied with each kit. Adding to this problem, the change of dye in the Phadebas reagent also caused a change in the assay sensitivity.

To meet the demand from labs analysing honey , Magle has developed a product that mimics the behaviour of the batch used in the original study. The product, which will be released later this year under the tradename Phadebas Diastase Test (PDT), has been developed in accordance to Magle's quality management system (ISO 13485 and pharmaceutical GMP). The result is a product with very narrow specifications wherein all batches of PDT will behave alike and adhere to the set of equations in the IHC guidelines. PDT will be launched as an improved and cost effective alternative to the Schade method with: better precision, fewer reagents, fewer steps and less labour time. This presentation will discuss the quality assurance program as well as present the new product and the company.

BIPEA - ORGANISER OF PROFICIENCY TESTING SCHEMES FOR HONEY

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Created in 1970, BIPEA is an international non-profit organization of testing laboratories. BIPEA regularly organizes 56 Proficiency Testing Schemes (PTS) in the following fields of interest:

- Cereals, oilseeds and animal feed
- Food industry and beverages
- Environmental issues (quality of water) and contaminants
- Cosmetics and fragrances.

BIPEA missions are:

[1] Harmonised Methods of the International Honey Commission (http://www.bee-hexagon.net/files/file/fileE/IHCPapers/IHC-methods_2009.pdf)

- To organize inter-laboratory comparisons tests for the laboratories to check their performance.
- To assist laboratories in the management, maintenance and improvement of their performance.

For a laboratory, the participation in a P.T. Scheme is a tool for self-checking and allows:

- Cntrol of the accuracy of a result of the laboratory analysis
- Quantification of the bias of the laboratory.

Since 2001, BIPEA has organized a Proficiency testing scheme for honey: five times per year, the laboratories receive homogeneous samples of honey. Thus the laboratories are given the opportunity to compare themselves, anonymously, not only for various physico-chemical parameters, but also for microscopic examinations (floral and geographic origins), sensorial analysis and honey identification. With 30 international participants, located all around the world, we adapt our program to the different kinds of products that can be found in these different countries in order to meet their needs.

PARTICIPATION RESULTS OF APICULTURE DIVISION LABORATORY IN PULAWY (POLAND) IN PROFICIENCY TEST FOR HONEY ORGANISED BY BIPEA

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Bee Products Quality Testing Laboratory has participated in proficiency test organised by Bipea (Bureau InterProfessionnel d'Etude Analytique) since 2004. In 2008/2009 campagne the laboaratory participated in all five rounds and received satisfactory results (except sucrose content in September 2008) for the accreditated methods of honey quality determination: moisture (refractometric method), pH and free acidity (potentiometric method), electrical conductivity, 5-hydroxymethylfurfural (with HPLC and after Winkler), diastase activity (with Phadebas), sugar content (with HPLC) and pollen analysis.

Table 1. Results estimation obtained by Bee Products Quality Testing Laboratory in interlaboratory comparisons in 2008/2009 campagne.

Measurement parameters	Z-score* obtained for each parameter				
	Round 1/ September 2008	Round 2/ November 2008	Round 3/ January 2009	Round 4/ March 2009	Round 5/ May 2009
Water content	0,3	0	0	0,5	0,5
pH	1	2	1	1	1
Free acidity	0,5	0,1	0,8	0,4	0,5
Electrical conductivity	0,6	0,2	0,5	0,8	0,1
HMF content	1,3	0,3	0,7	0,9	0,2
Diastase activity	1	1	1,25	1	0,5
Sugar content					
Fructose	0,9	0,4	0,05	0,2	0,2
Glucose	1,4	0,1	0,5	0,7	0,25
Sucrose	2,2	-	-	-	1,3
Maltose	0,4	0,9	0,5	1	0,5
Turanose	0,2	0,3	0	0,5	0
Trehalose	-	1,5	0,7	1,4	-

* **z-score = $(X_{lab} - X_{tot})/S$**, (x_{lab} – laboratory result; X_{tot} – mean from all quantified results; S – standard deviation with all quantified results. Interpretation criteria of z-score: $z \leq 2$ – satisfactory result; $2 < |z| \leq 3$ – doubtful result; $|z| > 3$ – unsatisfactory result.

Participation in interlaboratory comparisons is one of the few way used in Bee Products Quality Testing Laboratory for quality control and allows maintaining a high level of proficiency of the accreditated methods there.

A REVIEW OF THE BIOLOGICAL AND FUNCTIONAL PROPERTIES OF HONEYDEW HONEYS IN COMPARISON TO BLOSSOM HONEYS

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Honeydew honeys and blossom honeys differ in regards to different physico-chemical properties. In comparison to blossom honeys honeydew honeys, have generally a darker colour, higher electrical conductivity, higher pH, lower amount of fructose and glucose linked to a higher amount of oligosaccharides, and higher concentration of phenolics.

In one study the glycemic index of honeydew honey was found to be higher that that of different blossom honeys (Berg et al. 2008).

The prebiotic effect of honeydew is expected to be higher than that of blossom honeys due to its higher content of oligosaccharides, thought to be the principle prebiotic components (Sanz et al. 2005).

The antioxidant effect of honey depends mainly on the phenolic conent of honey. Dark honeys, and also honeydew honeys, have a higher content of phenolics and their antioxidant activity is higher than that of light blossom honeys. Oak honey, the darkest honeydew honey seems to have the highest antioxidant activity of all honeydew honeys (Bobis et al. 2008).

The antibacterial activity depends on the botanical origin of honey. Honeydew honeys have been reported to have generally higher antibacterial activity than light blossom honeys (Molan, 1991). This difference seems to be true both for the peroxide and non-peroxide antibacterial system.

On the other hand, the health enhancing properties of honeydew and blossom honeys have not been the object of comparative clinical studies.

FIRST ESTIMATIONS FOR THE CHESNUT HONEYDEW TYPIFICATION IN SPAIN

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SPAIN

Castanea sativa Miller presents a great interest for honey production in Northern and some regions of Central Spain. The bees obtain from it an important quantity of pollen, nectar and frequently honeydew.

Chestnut honeys present some sensorial characteristics very appreciated by a good number of consumers, as its dark amber colour sometimes with reddish tone; its strong, durable and woody olfative perception and its sweet-salted flavor with certain bitter aftertaste. Astringency is other common mouth perception. Some of these aspects are common to honeydew honey. The same occurs with some physico-chemical characteristics: high electrical conductivity, color, pH, etc.

The aim of this work is to analyze honeys with similar sensorial attributes and differentiate their source: nectar or honeydew. We study spanish honeys from different geographical origin, where Chesnut honey is typical, like Galicia, Villuercas-Ibores (Extremadura) and Montes de Toledo (Castilla-La Mancha).

Taking into account physico-chemical parameters as: electrical conductivity, HMF, diastase, pH, free acidity, moisture, and other like sugars or palynological analysis.
Main differences between floral honey and honeydew were observed in electrical conductivity and sugars contents.

This work has been financed by Junta de Comunidades de Castilla-La Mancha (proyect PAI09-0018-9267 and predoctoral scholarship).

COMPARISON OF VOLATILE AND SEMI-VOLATILE COMPOUNDS ISOLATED FROM SIX TYPES OF GREEK MONOFLORAL HONEYS

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Volatile compounds contribute to consumers' preferences and can also be used as a useful tool for the determination of honey's botanic origin. In this work the volatile profiles of six types of Greek monofloral honeys were determined using a Purge & Trap – Gas Chromatography – Mass Spectrometer system. A total of 166 compounds were detected in all samples, 81 of them in pine, 95 in oak, 83 in fir, 72 in knotgrass, 85 in Erica and 71 in thyme honeys. Twenty eight substances were detected in every sample independently of their origin, of which the most important were octane, nonane, benzaldehyde, phenylacetadehyde, nonanal and decanal. In total, 86 substances were isolated in all samples at least at one type of honey and these compounds used to form characteristic combinations for each kind of honey and for the statistical analysis as well. Some substances were isolated only from all samples with the same origin, like 2,3-dihydro-1,8-cineol and α-calacorene from oak honey, methyl-benzyl-methanol from fir honey, 2-hydroxy-isophorone and α-ionone from Erica honey. The characteristic compounds and combinations can be used together with the physicochemical microscopic and organoleptic characteristics in order to determine the botanical origin of the studied Greek honeys.

INTERPRETATION OF INDIAN UNIFLORAL HONEY QUALITY DATA BY PATTERN RECOGNITION TECHNIQUES

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In this study, it was attempted to determine the quality variables capable of promoting a characterization of the honey from different plant sources viz. *Trifolium alexandrinum (berseem clover), Brassica sp. (mustard), Helianthus annuus (sun flower), and Eucalyptus lanceolatus. A*mong the sources of honey significant difference were found in moisture content, free acidity, pH, fructose glucose ratio, ash content, diastase activity, hydroxymethylfurfural content and mineral content (Potassium, sodium, calcium, zinc and copper). Pattern recognition techniques such as principal component analysis (PCA) and linear discriminate analysis were performed to classify honeys according to their type on the basis of physicochemical parameters and mineral content. The variables with high discrimination were potassium, sodium and total acidity.

SLOVENE HONEY - *PROTECTED GEOGRAPHICAL INDICATION*

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SLOVENIAN

Around 500 honey samples, representing various floral and honeydew honeys, were collected over four consecutive harvest seasons from various areas of Slovenia. The following physico-chemical properties were tested: electrical conductivity, acidity, diastase number, water content, insoluble solids, HMF, fructose, glucose and sucrose and also residues of acaricides. It has been shown that most honeys lie within the limits set by legislation and most of those lie far below the limit. According to these results, the Slovenian Beekeepers Association decided to get a *Protected Geographical Indication (PGI) for honey produced in Slovenia and to respect all required parameters (honey quality, pollen spectrum, GMP, etc.).*

UNI-FLORAL HONEY ANALYSIS COMMERCIALIZED IN CATALONIA (SPAIN)

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The characterization of 24 samples of Catalonian commercial brands of uni-floral honeys was carried out on the basis of melissopalynological analysis, physico-chemical properties (colour, water content, HMF, diastase, pH, electrical conductivity and poly-phenols), enzyme analysis (diastase and peroxide), amino-acid test (proline) and residues from antibiotic treatments (tetracyclines). The botanical origins of the examined honeys were *Castanea sativa* Mill, *Citrus* spp, *Erica* spp, *Eucalyptus* spp, *Lavandula* spp, *Medicago sativa*, *Quercus* spp (honeydew), *Robinia pseudoacacia* L, *Rosmarinus officinalis*, *Thymus vulgaris*, *Tilia* spp and multifloral honey. Pattern recognition methods such as principal component analysis (PCA) were performed to classify honeys according to their type on the basis of physico-chemical parameters. These combinations of methods could be a promising approach to prove authenticity of honey.

BEEKEEPING RUSSIA

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By this time in 72 territories of the country engaged in beekeeping there is about 5 thousand households and about 300 thousand individual beekeepers, farmers and cooperators. Bees are placed unevenly by region, their numbers ranged from 0.2 to 259.9 thousand.

In 48 territories of 72 the number of bees is less than 50 thousand and an average of one region here has to 24,4 thousand in 17 territories, there are 50 to 100 thousand bees, the average of this group is 71,7 thousand. And only 7 territories of Russia have a high saturation, reaching 177.8 thousand bees per 1 region.

The number of bees during the years of reform during 1991-1995, decreased from 4501.6 to 3741.3 thousand, or almost 17%. In the next years, the dynamics of reducing the number of bees survived, but its rate decreased significantly. Over the 1996-2000 periods, Russia lost almost 300 thousand bees, or 8%. In 2001, the decline in the number of bees continued.

A particularly sharp reduction in the number of bees observed in the public sector. From 1991 to 2001 the number of bees in the former and state farms has decreased from 1730.7 to 526.6 thousand, or 3.3 times.

However, it should be noted that in the garden sector over the years of reform, there is no recession, and growing numbers of bee colonies from 2,770.9 in 1991 to 2,914.9 thousand in 2001 or 10,5%, it mean that individual beekeepers have managed to survive in difficult conditions of restructuring the economy of the country.

Despite the significant decrease in the number of bees, the volume of commercial honey production has not decreased, but increased from 48.4 thousand tons in 1991 to 52,6 in 2001, or 10,9%. Depending on weather conditions and quality of winter this figure over the past 10 years ranged from 43.9 thousand tons in 1994 to 57.7 tons in 1995.

The growth of commercial honey in the country due to the fact that in the garden sector, where an increase in the number of bees, productivity by 60% higher than in the apiaries of public economies.

During the reform period the number of bees not only survived, but only increased in farms of the Central Black Earth Region (400.9 thousand in 2001 against 356.7 thousand in 1991) and the Kaliningrad region (55.8 to 50 , 5 thousand, respectively). At the same time in the Far Eastern economic region of 470 thousand, available in 1991, remained 134,0 thousand it mean that their number has decreased in 3,5 times. Positive shifts are observed in beekeeping Republic of Bashkortostan, Chuvash Republic, Perm and Sverdlovsk regions. This increase honey production compose to 200-400%.

VOLATILE AND SEMIVOLATILE COMPONENTS OF *Mesembryanthemum crystallinum* HONEYS FROM GRAN CANARIA (SPAIN)

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As a preliminary study for the characterization of *Mesembryanthemum crystallinum* honeys from Gran Canaria (Spain), we have analyzed the profile of volatile and semivolatile compounds of six samples. Aroma-compounds isolation was performed by solvent (ethyl acetate) extraction and the concentrated unmethylated extracts were analyzed by GC-MS. In the samples analyzed 71 compounds were found, the most important of which are the following: Among aliphatic compounds: levo and *meso*-butane-2,3-diol isopropanol and 1,3-dihydroxy-2-propanone. Among monoterpenes, both isomers *E* and *Z* of 2,6-dimethyl-2,7-octadien-1,6-diol were found. Among benzene derivatives: 3,4,5-trimethoxy-benzoic-methyl-ester was found as a major component in most samples. Other benzene derivatives found as important components were 4-vinyl-2-methoxy-phenol and phenylacetic acid. Many Norisoprenoids were also found being the most representative vomifoliol. Among compounds related with heating or degradation processes HMF, 2-hidroxy-2-ciclopenten-1-one and 2,3-dihydro-3,5-dihydroxy-6 methyl-4-H pyranone were found. Finally, caffeine was found in all samples with area percentages ranging from 0,53% to 7,72%.

EUCALYPTUS OCCIDENTALIS ENDL. UNIFLORAL HONEY: PHYSICOCHEMICAL, MELISSOPALYNOLOGICAL AND ORGANOLEPTIC CHARACTERISTICS

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In Italy Eucalyptus unifloral honey is mainly produced on *E. camaldulensis* Dehnh.; its analytical profile is well known and currently used for authenticity control of the unifloral denomination. In the last years we observed some honey batches, marketed as Italian Eucalyptus honey, that do not fulfil the required outline, mainly for the melissopalynological characteristics. The productive information reveals that these products originate from another *Eucalyptus* species, blooming in late summer - early autumn, *Eucalyptus occidentalis* Endl. . A study was then carried out on about 50 samples of this kind of honey, purchased directly from the producers, in order to describe its physicochemical, melissopalynological and organoleptic characteristics. The results showed that physicochemical and organoleptic characteristics are quite

similar to those of *E. camaldulensis* unifloral honey, except for sucrose content, slightly higher in *E. occidentalis* honey. On the contrary, melissopalynological features, with an absolute pollen number (PG/10 g) between 20.000 and 50.000 and a relative frequency mainly between 40 and 80%, are specific of this species, also characterized by bigger pollen grains. Mixed honeys from both Eucalyptus species, with intermediate features, are quite frequent.

LEAD AND CADMIUM IN HONEY DEW AND BLOSSOM HONEYS ORIGINATING FROM DIFFERENT REGIONS OF GREECE IN THE YEAR 2009

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Lead (Pb) and Cadmium (Cd) are considered the principal toxic heavy metals of environmental contaminants. The proposed to the E.U MRL (Maximum Residue Limit) (Byrne,2000), is 0.1mg/kg for Cd and 1mg/kg for Pb in food products.

In the present study, 22 honey samples (14 honeydew, 5 blossom honeys and 3 blossom and Honeydew), originated from different parts of Greece, were examined. These samples come from the annual national residue program for the honey, of the year 2009..
The method used was from our lab of Residue Analysis in food of animal origin and the samples were determined by atomic absorption spectrometry.
The LOQ for Pb was 0.0016 mg/kg and for the Cd 0.00055 mg/kg. .
For Pb in the honeydew honeys the measurements ranged from 0.0021 mg/kg to 0.055 mg/kg. and all values were found below the proposed limit of 1mg/kg..MRL (Maximum Residue Limit), whereas for Cd the measurements ranged from below the LOQ (Limit of Quantitation) of the method to 0.0014 mg/kg, with all the values below the proposed limit of the MRL 0.1 mg/kg.
For blossom honeys, for Pb the measurements ranged from 0.0032 mg/kg to 0.018 mg/kg and all values were found below the proposed limit of 1mg/kg, whereas for Cd the measurements ranged from below the LOQ of the method, with all the values below the proposed MRL limit of 0.1 mg/kg.
In the 3 blossom and honeydew honeys the measurements ranged from 0.0093 up to 0.070 mg/kg for Pb and for Cd the measurements were found below the LOQ of the method. All values were found below the proposed MRLs.
All values were found very low and it is noticed the relatively low concentration of honeys of Pb which is probably due to the increased use of car-engine catalysts.

HONEY BEES OF WESTERN TURKEY AND PINE HONEY

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Turkey holds more than 20% of all known honey bee species in its geography. Among these *Apis mellifera anatoliaca* predominantly distributed all over the geography. *Apis mellifera caucasica* has its own geography naturally in north east of Turkey along the border to Georgia. *Apis mellifera meda* is distributed in the south east of Turkey on the border to Iraq and Iran. *Apis mellifera syriaca* is found on the southern Turkey bordering to Syria. Honey bees of Thrace are different than other bees diagnostic to carnica group.

The bees of western Turkey according to Ruttner (1988) is found different than the rest of Turkey and associated with the pine honey. Here we studied the new methodology in order to distinguish these bees with using reference samples. We found that the bees from this region is statistically different ($P<0.05$) than the subspecies distributed from the other parts of Turkey based on geometric morphometric methods of both types landmark based and shape analysis of wing cells. Here we report the honey bee differences, beekeeping in western Anatolian and the economics of the region on Turkish beekeeping sector.

PINE AND FIR TREE HONEYS: A REVIEW OF THE TWO PILLARS OF BEEKEEPING IN GREECE

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Pine and Fir honey provide more than 70% of Greece□s annual honey production. We will present important facts about these two major honey sources, the plants themselves, their insect pests that provide these valuable honeydews, focusing at the beekeeping techniques involved to exploit them. For fir honey, "early split and unite" method is widely used by Greek beekeepers. This consists of splitting the colony to two equal parts in early spring (mid March) and uniting them again right after moving them to fir forests (end of May). In such way, 4-super hives are formed, with excessive number of forager bees. Problems concerning fir honeydew are lack of space (which leads to spread of diseases, robbing, drifting), lack of water in the apiary, and presence of bears that thrive in these regions. About pine honey, bees need extra pollen supplies, since they come out of summer harvests, lacking new worker bees and capped brood. They are either transferred to special plants that provoke brood raising (such as heather, ivy, heliotrope, etc) or they are given pollen patties, made in autumn by the beekeepers themselves. Problems that Greek beekeepers have to solvewhile in pine honeydew are danger of summer fires, lack of space, lack of pollen & water, severe attacks from wasps and hornets.

Pine and Fir honey provide more than 70% of Greece's annual honey production. We will present important facts about these two major honey sources, focusing on the plants themselves, their insect pests that provide these valuable honeydews, as well as the beekeeping techniques involved to exploit them. Special interest will be given to Vanilla-Fir Honey from Mt Mainalo of Peloponnesus, the only Greek honey certified as to its origin (PTO – Protected Designation of Origin)

PREDICTION OF 16 PHYSICOCHEMICAL MEASURANDS OF HONEY USING FT-IR ATR

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Fourier Transform Infrared spectroscopy with an attenuated total reflection accessory (FT-IR ATR) was used to predict 16 measurands in honey. 416 different honey samples obtained from the Swiss National Honey Quality Program of the years 2006 and 2007 were analyzed by classical physicochemical methods and FT-IR ATR. Partial least squares regression (PLS) was used to develop the calibration models for the measurands studied (electrical conductivity, erlose, free acidity, fructose, fructose/glucose ratio, glucose, glucose/moisture ratio, HMF, isomaltose, , melezitose, moisture, pH, sum of fructose + glucose, , total nitrogen, trehalose and turanose) They were validated using independent samples and proved satisfying accuracies for the determination of electrical conductivity, melezitose, and moisture. Poor predictive quality was found for erlose, fructose, HMF, isomaltose, total nitrogen, trehalose, and turanose while the calibrations for the remaining measurands can be applied for rough screening purposes. The results showed that mid-infrared spectrometry can be used as a screening method for the routine analysis of some quality characteristics of honey with the advantages of being rapid and non-destructive.
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CHROMATOGRAPHY ANALYSYS OF CARDOHYDRATES USED IN QUALITY CONTROL OF HONEYS

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Honey is a natural sweet substance composed mainly of easily digestible simple sugars and of some small percentage of di- and trisaccharides. The qualitative and quantitative composition of sugars is peculiar to the honey. Chromatographic spectrum of honey carbohydrates is guarantee of the product authenticity. The aim of the study was comparison of sugar composition of honey and several syrups (bee feeds) before and after they were processed by bees. Another objective was to find some significant differences between the bee-processed syrups and honey. According to the received results the differences were determined for fructose content, fructose-to-glucose ratio, and within disaccharides, for sucrose and maltose content. Fructose content below 32%, maltose content over 5% and fructose-to-glucose ratio (F/G) below 1 can be used as a distinguisher of honey adulteration with winter stores processed from starch syrups. For identification of inverts processed by bees from sucrose syrups, erlose content (over 2%), sucrose content (over 3%) and sucrose-to-maltose ratio (approaching 1 or higher) can be used.

HPLC DETERMINATION OF MALTODEXSTRINS USED FOR DETECTION OF HONEY ADULTERATION BY STARCH SYRUPS

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For several years in Poland various types of starch syrup have been produced and utilized chiefly by food industry. Some of those syrups have roused the interest of beekeepers due to their relatively low price and convenient ready-to-use form. The aim of the study was to develop a quantitative assay method for maltodextrins **occuring in starch syrup** with an idea to use that method for identification of honey adulteration with admixtures of those syrups. The experimental material was comprised of starch syrups and winter stores processed from those syrups. Moreover, honey samples with an admixture of starch syrups prepared in the laboratory were analyzed. Separation, identification and quantity analysis of maltodextrins **(**maltotetraose - Dp4, maltopentaose - Dp5, maltohexaose - Dp6 and maltoheptaose - Dp7) **were done by Shimadzu HPLC with** refractometric detector. Chromatographic conditions were performed on Phenomenex Luna 5µm NH_2 100A 250 x 4.60 mm column at a temperature of 40°C with the mobile phase of 65 : 35 acetonitrile-water and 3 ml/min flow rate. The quantitative assays were made using the external standard method. The detection limit for all maltodextrins was calculated on 0.05 g/100g. The precision and repeatability of the method were satisfactory. The correlation coefficient of linear dependence of malto-compounds concentration on the peak area received value over 0.999. Maltodextrin content in starch syrups and in the winter stores processed thereof was 3.3 and 2.3 g/100g, respectively. **The elaborated method for maltodextrin assay** can be used for estimation of honey adulteration with winter stores processed from starch syrup or with the syrup per se. The method allows the detection of 10% addition of starch syrup in honey.

DEVELOPMENT OF A SCREENING METHOD FOR THE AUTHENTICATION OF THE BOTANICAL ORIGIN OF HONEY BY MID-INFRARED SPECTROSCOPY

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Fourier transform mid-infrared spectroscopy (Tensor 27, Bruker) using an attenuated total reflectance cell (ATR-MIR) was applied to model a spectral-library basing on the spectral characteristics of 6 different unifloral honey types (acacia, rape, lime, heather honeydew, n = 239) previously classified with official methods (chemical, pollen and sensory analysis).
The IDENT-method (OPUS 6.5, Bruker) was developed after multivariate data analysis and selection of significant spectral ranges (wave number range between 4000 and 550 cm-1). The developed IDENT-method (spectral library) consists of one headlibrary and 4 sublibraries on 3 different levels. The IDENT-method was tested for 72 honey samples, so far. Most of the unifloral honeys revealed very high rates of

correct classification (Acacia 100%, Heather 92%, Lime 100%, Rape 97%, Honeydew 71%). The results demonstrate that the model used was robust.
Before this new method can be used for routine honey control, it has to be tested with a sufficient number of authentic unifloral samples. The range of the method has to be amplified for more honey types, unifloral and polyfloral.

SOLID-PHASE EXTRACTION FOR THE DETERMINATION OF ORGANIC ACIDS IN HONEY

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Even though organic acids represent less than 0.5 – 2.0 % of honey [1, 2, 3], they are significantly involved in the organoleptic, physical and chemical properties of honey. Furthermore, aliphatic organic acids were used to characterize honeys according to their botanical and/ or geographical origin [4, 5].

A solid-phase extraction procedure was applied to isolate organic acids from honey. Acetic, citric, formic, fumaric, galacturonic, gluconic, glucuronic, glutaric, lactic, malic, oxalic, propionic, pyruvic, succinic and tartaric acid were extracted using an anion-exchange cartridge. Different clean-up steps such as the conditioning and the elution parameters which influence the recovery of the acids were studied and consequently optimized. With the final method the average recovery of the 15 organic acids in honey was above 90 %. The organic acids were determined via HPLC-DAD at 210 nm.

As expected, honeydew honeys contained the highest amount of total organic acids with 11.2 g/kg on average. The results of this research will be presented and discussed.

SIMULTANEOUS ANALYSIS OF PHENOLIC ACIDS AND FLAVONOIDS IN HONEY

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Certain phenolic acids and flavonoids are suitable as marker substances to characterize several unifloral honeys. First results were presented by Speer und Montag (1984, 1987) and Steeg und Montag (1987, 1988). Several years later, the study-group of Tomás-Barberán (1992, 1995) reported further results concerning the flavonoids in honey. Up to date, a large number of investigations have been published about phenolic acids and flavonoids in honey.

As most of the authors applied different methods for extraction and determination, the obtained data could, frequently, hardly be compared and showed remarkable discrepancies concerning the qualitative and the quantitative data.

Hence, a fast and easy extraction method for both the phenolic acids as well as the flavonoids had to be developed. The separation and detection was, then, carried out using HPLC-DAD and UPLC-Q/TOF-MS. Thereby, 34 substances were identified in the ethyl acetate extracts of sunflower, lime, clover, rape, and honeydew honey.

ANALYSIS OF THE VOLATILE FRACTION COMPOSITION IN THE BEE POLLEN LOADS IN SPAIN

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The bee pollen is one of the beehive products that require a complete study to obtain its characterization as a quality product and its identity appreciation on a botanical profile. Thus, the study of volatile fraction has been carried out. It is known that pollen aroma is determined by the floral origin of the pollen, but it can vary after the drying process required for its marketing.

As a result of these premises, the study of 13 samples of bee pollen was done. Analysis results from several subsamples that were dried out pollen at different temperatures were compared with fresh pollen.

The aim of this study was the detection of the volatile compounds that can identify its floral origin, as well as those that can reflect the conditions related to the drying process of the pollen. This information was used to establish which dryness temperature was critical for the aroma loss of the pollen.

Pollen was dissolved in an ethyl acetate solution for the extraction of the volatile compounds and then analyzed by a gas chromatography / mass spectrometry. 146 aromatic compounds have been found and statistically analysed through a discriminant analysis, in order to identify and detect which ones are related to each dryness temperature. Final evaluation reduces the markers compounds to a 17-27 range. Among them, 16 compounds can be used as a tool to identify botanical origin and dryness temperature.

This work has been financed by INIA-FEDER project RTA2007-00072-C03 and a predoctoral scholarship financed by INIA.

ANALYSIS OF FRENCH ROYAL JELLY FOR QUALITY AND AUTHENTICITY CONTROLS

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The aim of our study is to characterize french Royal Jelly (RJ) in order to define a standard composition and evaluate the quality of commercial products.

More than 300 RJs produced recently in France were analyzed. Due to the important heterogeneity of the materials depending on the environment of the hives, the climate, the soil etc, the samples were collected in different french regions during the course of the harvesting season representing various geographical and botanical origins. All the samples analyzed for creating our data base were provided by beekeepers belonging to the GPGR (Groupement des Producteurs de Gelée Royale), a french cooperative that respect a quality charter concerning the production, the sampling procedures and the storage of the RJ.

We have developed and validated analytical methods to quantify various parameters : water, protein, 10-HDA, amino acid, sugar contents. Moreover stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were measured in RJ samples by isotope ratio mass spectrometry.

In comparison, around 60 commercially available RJ samples, as representative materials produced and traded worldwide, were analyzed by the same methods.

In addition, around 30 RJ from feeding experiments with artificial sugars were provided by some apiarists in order to evaluate the impact of sugar feeding on the composition of RJ.

This study has allowed to establish ranges of natural variation of different composition parameters in french RJ and to define criteria for genuine ones. Some parameters are essential to distinguish RJ produced in France from abroad RJ and/or from intensive feeding, included isotopic measurements of ^{13}C and the content of some particulate sugars.

THE CONTENT OF FATTY ACIDS IN MONOFLORAL POLLEN LOADS

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Contect of fatty acids as well as total lipid content in 7 samples of fresh monofloral bee pollen loads was evaluated in this study using GC/MS. Pollen loads were collected from bees using pollen traps when placed on monocultures of rape (*Brassica napus*), white mustard (*Sinapis alba*), sunflower (*Helianthus annuus*), poppy

(*Papaver somniferum*), apple trees (*Malus domestica*), willow (*Salix* spp.) and dandelion (*Taraxacum officinale*).

Highest total lipid content was detected in pollen loads from rape (13,34%) and dandelion (11,63%). Dominant fatty acids in various pollen loads are linoleic, palmitic, tridecanic, linolic, oleic and capronic acids.

Fatty acids was hypothesised to improve honey bee longevity. Different concentrations of various fatty acids mentioned in this study can be used as a floral origin marker of pollen. This work has been supported by project VEGA 1/0074/08.

PROTEIN CONTENT AND SUGAR COMPOSITION OF BEE-COLLECTED POLLEN FROM SELECTED BOTANICAL ORIGINS

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The objective of the study was to investigate the protein content and sugar composition of honey bee-collected pollen. The pollen pellets were harvested with pollen traps, separated and classified according to their botanical origin. In 37 different botanical species the protein content was determined using the Kjeldahl method. The crude protein content in the examined samples ranged from 10,69% for Smilax pollen to 25,90% for that of Fallopia type. Mean value was 17.84%. Pollen from insect-pollinated species (*Castanea sativa* 21,42%) was richer in protein compared to that from anemophilous plants (*Pinus* 12,17%). Although honeybees posses the ability to discriminate among pollen types they are incapable of discriminating protein content.

The analysis of sugars was carried out by high-performance liquid chromatography. Differences were also found in the concentrations of the eight investigated carbohydrates (fructose, maltose, sucrose, trehalose, turanose, melibiose, melezitose, glucose) among various botanical species.

VITAMIN B_2 STABILITY OF DRIED BEE POLLEN DURING STORAGE

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Bee pollen has been used as a food supplement on account of its importance as a source of essential nutrients, among them, vitamins. Vitamin B_2 (riboflavin) has an important role in cell respiration, metabolism of proteins, fats and carbohydrates and also has participation in vitamins B_6, B_9 and B_{12} metabolism. Riboflavin is stable during food processing and storage. However, it is very sensitive to light and there is

a lack about bee pollen riboflavin values in literature. The aim of this work was to analyze this vitamin in seven samples of dried bee pollen commercialized in Brazil and to study its stability after eight months of storage in the original packages under three different conditions: room temperature (with and without light) and frozen. Riboflavin was analyzed by HPLC with fluorescence detection. The average content of this vitamin in time zero was 2.09 ± 0.32 mg/ 100g and the average contend after storage was 24.40%, 28.23% and 33.50% less in samples stored at freezer, room temperature without light and room temperature exposure to light, respectively. Statistically there is no variation ($p<0.05$) between the three storages conditions.
Acknowlegements: FAPESP, CNPq, USP

INHIBITION ACTIVITY OF ETHANOL EXTRACT OF PROPOLIS (EEP) RELATION MYCOTOXIGENIC FUNGI

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The contamination of dried fruits by mycotoxigenic fungi is a actual problem with economic, and health value.

Results of mycological analysis of dried fruits and raisin in Armenia , show their high contamination by ochratoxigenic and afltoxigenic fungi from *A. nigri and A. flavi* section. In recent years attention has been focused on the use of propolis as a health supplement suited to consumers in developed countries. Propolis, a natural honey bee product, has different biological activities.

The aim, of this study was to determine the effectiveness of ethanol extract of propolis (EEP), from some region and different concentration , relation samples of raisin with high level contamination by ochratoxigenic fungi and afltoxigenic fungi from *A. nigri and A.flavi* section. Antifungal activity also was studied, in vitro, by injection of certain quantities of extracts of propolis in Czapek-Dox agar. After solidifying, the disc (5 mm dia.) of the fungus culture was placed in the center of Petri dish. The inhibition percentage of mycelial growth of 10 strains of *A. carbonarius , A. niger, A. flavus* species, was determinated according to Singh (Singh et al., 1999). MIC (minimal inhibition concentration) was defined as the lowest concentration that did not yield visual growth. All experiments were performed in triplicate.

Along with EEP, during raisin processing (treatment of raisin), defined concentrations of mixture of propolis and hydrogen peroxide were used (some concentration). Duration of treatment varied from 5 to 20 minutes.

The EEP and mix with hydrogen peroxide has shown the stable antifungal effect relation ochratoxin A producing strains *A. carbonarius* , is in experiment in vitro , on pure culture (60%) and treatment of raisin (95%), which contaminating some species from *A. nigri* section. The EEP inhibition percentage of *A. flavus* mycelial growth was between 40% -60%.

The results obtained in this work could be of importance in the search of new natural sources bioactive compounds.

IS PORTUGAL A COUNTRY OF PROPOLIS DIVERSITY?

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Portugal geographical situation and therefore their floral diversity point towards the existence of typical propolis from temperate zones, where bud exudates of poplar trees (*Populus* species) are the main source of this honeybee product. With the exception of Madeira and Azores Islands, where the floral diversity could present significant differences, it would be expected to find a product rich in flavonoid aglycones (flavones and flavanones), phenolic acids and their esters, characteristic of *European* propolis. In order to clarify this issue, several propolis samples were collected along the country, including islands, and their colour, ashes, content of wax, phenols and flavonoids, as also their phenolic and volatile profile, were determined. Albeit this work seems endless, it is clear that as dipper we study each sample, more diversity we find. In fact, the first phenolic profile we analyse by mass spectroscopy,[1] a propolis sample with a significant poplar source, revealed the presence of 37 phenolic compounds, from which, seven were identified for the first time in propolis. More, the straight comparison of the phenolic profile obtained by HPLC for all samples, allow the identification of five distinct propolis phenolic extracts, with a correspondence diversity found as well in colour and odour. Although some samples show a typical odour of *Cistus* species, a common resinous plant in the east side of Portugal, the exact botanical origin seems another giant task to overcome. The volatile results play here a complementary source of information to achieve this purpose.
The bioactive properties of the phenolic extracts were also studied in terms of reducing power and radical scavenging effect. Once again, the differences between propolis samples were clearly observed.

[1] Falcão, S.I., Vilas-Boas, M., Estevinho, L.M., Barros, C., Domingues M.R.M., Cardoso, S.M. (2010). Anal Bioanal Chem, 396: 887-897. DOI:10.1007/s00216-009-3232-8.

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PARAMETERS THAT AFFECT IN PROTEIN AND SUGAR CONTENT IN ROYAL JELLY

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Several groups of bee colonies were made to produce royal jelly in order to investigate the variation among bee colonies, the effect of artificial sugar feeding and the kind of grafted larvae on the quality of the royal jelly collected. The royal jelly that was produced was harvested, placed into vials and stored at -18^{o}C until analyzed. In total, 145 samples were collected. The samples were analyzed using High Performance Liquid Chromatography and Kjeldahl methods for the determination of sugars and proteins respectively. The protein content in analyzed samples ranged from 11,0% to 17,1%. Using a mixture of acetonitrile and water as mobile phase a total of eight sugars were determined in royal jelly samples (fructose, glucose, sucrose, melezitose, maltose, trehalose, turanose and melibioze).

PHYSICOCHEMICAL AND PALYNOLOGICAL ANALYSIS OF BRAZILIAN *Apis mellifera* BEE AND STINGLESS BEE (*Tetragonisca.angustula)* HONEY

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Honey is considered as a food that provides energy, being elaborated from the dehydration and transformation of the nectar of the flowers by the bees. For the human consumption, honey needs to attend the minimum requirements of identity and quality demanded by the regulation. In Brazil beekeepers can be divided in two practical distinct ones: the traditional ones, which use *Apis mellifera* bees and the Meliponiculture which uses native stingless bees such as "jataí" bee (*Tetragonisca angustula*). There are no identity and quality parameters or regulation for this type of honey. Honey from stingless bees is more expensive compared with the traditional honey; however it is commercialized without a proper regulation. The objective of the present work is to compare the composition and quality of honey from *Apis mellifera* and from *Tetragonisca angustula* bee from six cities of the state of Sao Paulo (southeast region of Brazil). The honey samples were obtained from both bees in the same botanical region. From botanical analysis it can be state that the honey from stingless bees present a bigger botanical diversity compared with Apis honey. It was used the methods based on the Brazilian Regulation for quality control of honey from *Apis mellifera*. The results for honey from *Apis mellifera* honey and stingless bee honey were respectively: moisture (15.40 -19.00 ; 23.40 -25.60 %), acidity (16.82-

32.47; 21.65 - 63,85 mEq/Kg), reducing sugars (52.98 – 84.24; 44.78 – 67.54 %), apparent sucrose (0,56 –7,64; 0,43 –4,46 %); total sugars (53.54 – 83.75; 45.57 – 67.68 %), HMF (2.00 – 21.00; 0.30 – 0.93 mg/Kg); diastase number (2.20 – 11.49; 11.01- 22.45); insoluble solids (0.01 – 0.08; 0.02- 0.10%) and ashes (0.11- 0.26; 0.17 – 0.42 %).

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VALORISATION OF THE HONEY FOOD CHAIN: TRACEABILITY AND BOTANICAL ORIGIN

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Traceability, Quality, Honey, Botanical and Geographical origin

Quality and safety of food products has always been a primary need in the food chain, in order to guarantee the consumer not only microbiological healthiness, but different qualitative aspects of all food. Quality is a multifactorial parameter linked to species and variety, agricultural methods and geographical origin.

For honey, one of the fundamental aspects of quality that affects its commercial value, is its botanical and geographical declaration of origin, as in its food chain, from harvesting to packing, the product should not have been submitted to any treatment that has modified its composition. Nowadays the diagnostic methods to determine the botanical and geographical origin are based on the sensory, microscopic and physico-chemical analysis.

In this work, analytical approaches of molecular traceability have been developed with the aim of establishing the botanical species in products from bees.

The use of methods of analysis based on nucleic acids allows a greater flexibility, due to the variety of the methodological approaches, as well as the robustness, speed and reproducibility of the process. DNA can be extracted from a variety of matrixes, is stable to technological treatments and therefore is particularly suitable for traceability in the process chain.

The Real-Time PCR tests allow for the identification, in a precise manner, of the species, variety and the place of origin of the food product.

Furthermore, the metabolic profile of honey has been defined by NMR spectroscopy. The aim was to identify the low molecular weight components, i.e. metabolites,

responsible of specific quality features, such as the botanic species, any treatments undergone and the area of origin.

THE BEE PERGA-ECOLOGICAL FOOD AND VALUABLE PROTEIN CONCENTRATE OF PATIENTS SUFFERING FROM ANDROID OBESITY AND METABOLIC SYNDROME

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Bee pollen is male sexual cells of blooming plants. Pollen seeds are invisible with the naked eye. Their size varies from 2 to 250 microns. Pollen seeds are living cells preserved from the hostile environment by a cover called exzine.

Perga is a bee product which is stored in the cells of honey-combs. It is used in medical practice as food, medicine, for disease prevention .

Two studies over patients suffering from android obesity were conducted. A three-month course of perga was carried out. The patients reduced their food taking to 1200ccal per day. The two main indicators-waist/hip ratio and BMI/body mass index/were observed. Twenty grams of perga, equalled to 49, 20 ccal, was added to the breakfast at ten a.m. Waist/hip ratio and BMI was measured every two weeks.

The aim of the second study was to estimate the prega effect on BMI and the cholesterol.One-month course was carried out. Twenty grams of perga were taken by patients two times a day in the morning and in the night.

During both of the experiments the patients haven`t taken any fibrates or statins. Patients suffering from type 2 diabetes were on their normal therapy.

The study showed decrease in cholesterol, triglyceride and basal glucemia.

Obesity is a complex, multifactor disease. It has connection with a series of factors such as social, psychological, behavioral and metabolic. Android obesity leads to metabolic and blood-vessel disoreder –insulin resistance and following diabetes, hyperprieteinemia, hypercholesterolemia, high blood pressure, metabolic syndrome, /syndrome''X''/ and following aterosclerosis. So reducing body weight to normal BMI is the way to overcome metabolic and blood-vessel complications in this specific type of obesity. The treatment with bee pollen and perga shows decrease in body mass to the normal values of BMI. This is a basic therapeutic method of prediabetes diabetes treatement.. A significant correlation between improvement of lipid profile and reduction of the risk of blood-vessel diseases has been found.

Perga with its quality to decrease lipid metabolism disorder is a vital part from food regime for improving the standard of life for these people. This is the way to broaden the boundaries of the complex treatment of android obesity and metabolic syndrome.

NON-AUTHENTIC ENZYMES IN HONEY

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There are present several authentic enzymes in honey. Natural origin of these enzymes may be nectar, pollen, bees, or even producers of honeydew.
We have met unusual property of the enzyme activity in some honey samples from markets recent years. We have found problems with determination of diastase activity in some market honey samples suspected for adulteration. Results obtained using Phadebas method were significantly different to results of Shade method. We suspect adulteration of diastase number by adding foreign diastase into market products, declared as honey.
The enzyme activity of several honey samples exceeds markedly natural range of enzyme activity in an authentic honey.
The other unusual enzyme in honey is special type of β-fructofuranosidase (invertase). This type of enzyme is absent in authentic honey, but is present very often in market products, declared as honey. The amount of this type β-fructofuranosidase in this suspicious samples is probably huge, because the activity of this enzyme is over limits of quantification.
The enzyme β -fructofuranosidase is used for industrial preparation of invert syrups. This enzyme is not authentic in honey. We suppose that market products declared as honey with such extra high activity of β-fructofuranosidase is adulterated by industrially inverted syrup.

IDENTIFICATION OF AROMATIC PATTERNS BY ELECTRONIC NOSE AND MULTIVARIATE ANALYSIS OF COLOMBIAN STINGLESS BEE HONEY

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Fifty three samples of Colombian stingless bees honey corresponding to genus *Mellipona* and *Trigona* from 2 different geographical regions were analyzed with an electronic nose. Stingless bees are known traditionally by indigenous population and recognized by sensorial and therapeutically properties of their products. The electronic nose was used to generate a pattern of volatile compounds present in honey samples in order to identify aromatic patterns which allow assessing the suitability of this instrument for discriminating among honey from different geographical origins. Data were evaluated by Principal Component Analysis (PCA); Discriminant Function Analysis (DFA) and Hierarchical Cluster Analysis (HCA). It was found that the main groups of volatile compounds responsible of aroma are those with a ring structure, short chain aliphatics, alcohols and organic sulfur substances. The results were also compared to data obtained from the volatile analysis of 53 samples of honey of the specie *Apis mellifera* of the same geographical regions; the multivariate analysis allowed to differentiate the samples of stingless bee honey and *Apis mellifera*. The obtained results suggest that electronic nose is a useful tool for the characterization of honey, which would allow searching differences that permit the establishment of Designation of Origin in order to add value to this product and enhance its popularity and demand.

EFFECT OF HEAT ON HONEY VOLATILE COMPOUNDS

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Honey is often subjected to heat treatments during postharvest processing. As a result, various alterations occur because of the destruction of some components and the formation of new ones. Considering the volatile fraction of honey, it is significantly altered when heat is employed. Various treatments were conducted with different types of honey in order to study the effect of heat on honey volatile compounds. In all cases, furan derivatives were the main class of components generated. Furfural concentration was significantly increased, while new derivatives were generated. The concentrations of phenolic compounds are generally elevated by heat, however severe conditions will destroy most of them. Moreover, certain compounds are favored when honeys of different origin were examined, meaning that the effect varies among honey types, depending on the physiochemical properties of each one. Also, the effect of procedures used to isolate volatile compounds from honey was studied. When using a method that significantly heats honey, the concentration of furfural is increased up to 15 times, while an array of new compounds are generated that contribute to the overall aroma. Phenylacetaldehyde is another compound that is favoured by these procedures; however its concentration in fact decreases when pure honey is heated. This is because the thermal generation of phenylacetaldehyde occurs in thermally treated honey solutions only. Conclusively, it can be pointed out that even mild heat treatment can alter the volatile composition of honey, thus changing the overall characteristic aroma.

VOLATILE COMPOSITION OF HONEYDEW HONEY FROM DIFFERENT GEOGRAPHICAL ORIGINS BY HS-SPME-GC-MS ANALYSIS

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Honeydew honey is increasingly valued by consumers because of its strong and characteristic flavor that is different from floral honeys. Such importance notwithstanding, the honeydew honey is so far not well investigated.

The volatile profile is one of the typical features of honey. The volatile's composition is specific for each type of honey and closely related to the geographical region of production. In order to characterize the volatile fraction of honeydew honey, HS-SPME-GC-MS and HS SPME-GC-FID analysis were performed. The identification of the volatile compounds were done with the aim to find marker compounds useful for the quality and authenticity control of the honeydew honeys. The studied samples were coming from three different geographical regions: Croatia, Slovenia and Austria. The results show a high number of volatile compounds. The compound's mean values range between 0.1-10.0 percent. Some of the compounds present in higher percentage are: benzaldehyde, n-nonane, α and γ-terpinene, benzeneacetaldehyde, *trans*- and *cis*-linalool oxide, n-nonanal, phenyl ethyl alcohol, 1-nonanol, n-decanal. Unfortunately it is not possible to highlight single volatile compounds that can be used as geographical markers, but all three groups of samples present characteristic volatile profiles. Due to particular geographical and botanical characteristics as well as climate conditions, a canonical discriminat function analysis (CDA) was applied to volatile compositions to distinguish honeys from Croatia, Slovenia and Austria. The territorial map obtained with CDA shows good discrimination between the honeydew honeys of the three regions. The two functions allowed a good classification of unknown sample (cross-validated). The high classification percentage indicates the possibility to apply the CDA using the composition of the volatile profile of honeys as a quality assurance tool enabling authenticity control.

Keywords: honeydew honey, volatile profiles, geographical markers, HS-SPME-GC-MS, CDA

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HONEYDEW HONEYS CHARACTERIZED BY THEIR ANTIOXIDANT ACTIVITY, FLAVONOID AND POLYPHENOL CONTENTS.

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European consumers can choose from nectar and honeydew honey, based on sensory perceptions. Honeydew honeys have also been characterized by chemical and microscopic analysis, to differentiate the floral or non floral origin of honey. In this work, a collection of honeydew honeys from all over the world was provided by the International Honey Commission to test bioactive properties such as the antioxidant activity and also the flavonoid and polyphenol content.

We followed spectrophotometric methods to measure the antioxidant activity AA (210.16 ± 154.34) μmol Trolox equivalents/100 g honey, flavonoid contents F (3.89 ± 1.37) mg quercetin equivalents/100 g honey, and polyphenol contents P (73.32 ± 31.51) mg gallic acid equivalents/100 g honey.

According to the geographical origin of the honeydew, the following values were achieved in mediterranean countries including Brasil (Brasil, Croatia, France, Greece, Slovenia, Spain): AA (247.58 ± 170.07), F (3.95 ± 1.39), P (83.99 ± 36.42) and others (Bulgaria, Czech Republic, Germany, Rumania, Slovak Republic, Switzerland): AA (168.81 ± 124.36), F

(3.84 ± 1.37), P (61,83 ± 19.95). This output shows that higher antioxidant activity is not explained by flavonoid and polyphenol contents.

According to the botanical and entomological origins, after ANOVA *post hoc* Scheffé, two groups of antioxidant activity and polyphenol content were detected, with higher values only for pine honey. No difference was found for the flavonoid content in all honey types. However, with the *post hoc* Duncan, more groups could be detected for polyphenols and flavonoid content, but still two groups differentiate the highest antioxidant activity of pine honey. Metcalfa is an intermediate group between pine and the rest of the honeys, for polyphenol content. Metcalfa and pine honeys showed significantly higher flavonoid contents than oak and spruce, with intermediate values for the rest of the honeys.

TOTAL FLAVONOID CONTENT OF HONEYS FROM BURGOS (SPAIN) AND ITS RELATIONSHIP WITH ELECTRICAL CONDUCTIVITY

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Flavonoids represent the most important group of honey phenolic compounds. They are currently receiving much attention because they can play a role as natural antioxidants, antibacterial, anti-inflammatory and vasodilator agents. Several relationships were described between flavonoid content and colour of honey. As colour is also related with electrical conductivity, because the darker the colour, the higher the electrical conductivity, the purpose of this work was to research if there was any relationship between total flavonoid content and electrical conductivity of honey. The analyses were carried out on 56 honeys from the province of Burgos (Spain). Total flavonoid content was measured by the Dowd colorimetric procedure. Flavonoids react with AlCl3 resulting in a yellow compound, whose intensity of colour varies depending on the flavonoid content of the sample. Total flavonoid content was very variable. Values ranged from 3.1 to 78.3 mg quercetin/100 g, with a mean value of 17.1 mg quercetin/100 g. 23% of samples showed values of total flavonoid content higher than 20 mg quercetin /100 g. In respect of electrical conductivity, 75% of honeys showed values lower than 0.8 mS/cm. At a 99% confidence level, we found a significant relationship between total flavonoid content and electrical conductivity (r= 0.7576) that would be worth studying in depth.
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COMPARISON OF ULTRASONIC SOLVENT EXTRACTS OF *SALIX* SPP. HONEYDEW AND NECTAR HONEY

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Among the compounds identified (ultrasonic solvent extraction followed by GC-MS), markers of the *Salix* spp. nectar honey botanical origin were phenylacetic acid, benzoic acid, 2-hydroxybenzoic acid, 4-hydroxybenzoic acid, 4-hydroxybenzyl alcohol and other compounds that originated from shikimate biosynthetic pathway, similar as for *Salix* spp. honeydew honey. All these compounds probably originated from *Salix* spp., a well known source of salicylic acid and its derivatives. High abundance of 3-methylpropanoic acid, 3-methylbutanoic acid, 2-methylpentanoic acid and 3-methylpentan-1-ol can be noted as specific for the nectar honey and several of them were found with lower percentages in the honeydew honey. The presence of pinocembrin and 8-hydroxy-4,7-dimethylcoumarin can be emphasized, since they were not identified in the honeydew honey. Phenylacetonitrile was present only in the nectar honey. Another group of abundant compounds were terepens, particularly linalool derived compounds. In comparison with the honeydew honey much more pronounced qualitative and quantitative composition of lilac aldehydes and alcohols were found. Norisoprenoids can be additionally considered as characteristic for the nectar honey, particularly 3-hydroxy-*trans*-β-damascenone, *trans*-β-damascone, 4-ketosiophorone, α-isophorone and others.

Keywords: *Salix* spp. honeydew and nectar honey, Volatile compounds, Ultrasonic solvent extraction, Gas chromatography–mass spectrometry

ANTIOXIDANT ACTIVITY OF HONEYS FROM BURGOS (SPAIN) AND ITS RELATIONSHIP WITH TOTAL PHENOLIC CONTENT

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Nowadays the determination of the antioxidant activity of honeys is a matter of significance. On the one hand, natural antioxidants are effective in preventing several diseases. On the other hand, honey can minimize deteriorative oxidation reactions in food commodities. Several researchers found relationships between antioxidant activity of honeys and total phenolic contents. Nevertheless, depending on the method emp loyed to measure the antioxidant activity, the correlations were considerably different. The purposes of this work were first, to determine in 56 honeys from the province of Burgos (Spain), the antioxidant activity by ORAC (oxygen radical absorbance capacity) method, as well as the total phenolic content by Folin Ciocalteu

procedure, and then, to find a possible relationship between these parameters. Almost the half of the honeys analyzed showed an antioxidant activity between 12 and 24 ORAC units (*µmol TE/g)*. With regard to the total phenolic content, more than 90% of samples exceeded the value of 20 mg gallic acid/100 g honey, whereas 30 honeys exceeded the value of 80 mg gallic acid/100 g honey. A statistically significant relationship (99% confidence level) between antioxidant activity and total phenolic content was found (r= 0.7587). After applying t-test, F-test, Mann-Whitney W-test, and Kolmogorov-Smirnov-test no significant differences were found between true and calculated antioxidant activities at a confidence level of 95%. If this correlation keeps in honeys from other geographical origins, the antioxidant activity of a given honey, whose measure is tedious and time consuming, could be calculated from its total phenolic content, therefore drastically reducing the experimental effort, with a significant saving of time and money.

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CHARACTERIZATION OF SIERRA MORENA (SOUTH SPAIN) *EUCALYPTUS* HONEY

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Characterization based on botanical, physicho-chemical and sensory analysis was carried out with the aim of establishing criteria for a future Protected Designation of Origin that includes *Eucalyptus* and other monofloral origins produced in Sierra Morena (geographical region in south Spain that covers the north of Andalusia). After the analysis of 41 samples provided from beekeepers as *Eucalyptus* honey, just 13 of these samples were set as homogeneous criteria probing their authenticable origin. Physicochemical parameters analyzed and criteria established were: water (≤17.5 %), pH (4.1-5.0), free acidity (≤35 meq/kg), electrical conductivity (0.38-0.58 mS/cm), color (40-60 mm Pfund), diastase (8.0-29.0 D.N.), glucoseoxidase (2.5-17.2-5 µg peroxide/g and hour) and hydroximethylfurfural (≤28 mg/kg). The botanical analysis confirmed minimum *Eucalyptus* pollen of 87% and Maurizio class III. Others pollens isolated were *Reseda, Echium, Trifolium* and *Cistus*. They were found two sensory profiles (five points scale) set as:

- Pale amber colour, a medium smell and taste intensity, (3) with hints of wood and vanilla and animal touches, low pastiness (1-2) and a very fine crystallization (1-2), a slightly salty taste (2), freshness, and a medium persistence (3).

- Yellow,(3), with a medium to high smell and taste intensity (3-4) with whiffs of liquorice, coffee and chocolate and a fruity taste of crystallized orange, a medium pastiness (3) and rough crystallization (4), a salty taste (2-4), freshness, spicy and a medium persistence (3).

CHARACTERIZATION OF SIERRA MORENA (SOUTH SPAIN) *ECHIUM* HONEY.

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Characterization based on botanical, physicho-chemical and sensory analysis was carried out with the aim of establishing criteria for a future Protected Designation of Origin that includes *Echium* and other monofloral origins produced in Sierra Morena (geographical region in south Spain that covers the north of Andalusia). 57 samples provided from beekeepers as *Echium* honey were set as homogeneous criteria probing their authenticable origin. Physicochemical parameters analyzed and criteria established were: water (≤17.5 %), pH (3.6-5.9), free acidity (≤35 meq/kg), electrical conductivity (≤0.5 mS/cm), color (≤60 mm Pfund), diastase (8.0-33.0 D.N.), glucoseoxidase (2.5-17.2-5 μg peroxide/g and hour) and hydroximethylfurfural (≤28 mg/kg). The botanical analysis confirmed minimum *Echium plantagineum* pollen of 85% and Maurizio class III or IV. Other pollen isolate was *Myrtus communis*. It was found a sensory profile (five points scale) set as:
White-very pale yellow colour; low smell and flavour intensity(2-3) clearly vegetal with balsamic touches of camphor and resin, woody hints, cooked vegetable and cabbage; sweet taste (3-4); low pastiness (1-2) and fine crystallization (1-2) and low-medium persistence (2-3).

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SPANISH LAVENDER (*LAVANDULA STOECHAS*) HONEY FEATURES IN SOUTH SPAIN.

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Honey formed from Spanish lavender (*Lavandula stoechas*), known in Spain as cantueso, is one of the uniflorals produced in Sierra Morena, South Spain. Its palynologycal, physicochemical and sensory characteristics were defined after a whole analysis process of 204 samples of honey with the purpose of establishing the criteria for a future Protected Designation of Origin (PDO). In this PDO, 7 different types of honey were described. Up to 10 of those samples were characterized as Spanish lavender honey, which features are described here.

From the palynologycal analysis it was concluded that the minimum *Lavandula stoechas* pollen percentage for this honey to be considered unifloral according to this

PDO must be 12%. *Echium, Myrtus* or *Reseda* can appear as the dominant pollen type. Taking into account its $PK_{10,}$ this kind of honey can be included either in II or III Maurizio class.

As regards the physicochemical characteristics, the colour in mm Pfund can vary from 35 to 55. The electrical conductivity ranges from 0.25 to 0.45 mS/cm. The diastatic activity varies between13 and 25 Gothe scale units.

Regarding its sensory characteristics it is established (five points scale) that it has yellow colour, low fluidity (1-2), low odour/aroma intensities (2-3) associated with floral and fruit notes respectively; medium sweet taste (3) and low persistence.

CHEMOMETRICS AND MINERAL CONTENT FOR THE IDENTIFICATION OF GEOGRAPHICAL ORIGINS OF SPANISH HONEYS.

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The mineral content of honey samples could give an indication of environmental pollution and herewith also an indication of the geographical origin of honey. Therefore, the objective of this work is to obtain a multivariate statistical model able to classify honeys according to their geographical origin with using the mineral composition and some of its physicochemical properties. Nineteen parameters were determined (colour parameters such us *x* and *y* trichromatic coordinates, dominant wavelength, absorbance net, turbidity electrical conductivity, pH, content of potassium, calcium, sodium, magnesium, manganese, nickel, lithium, chloride, nitrate, sulphate, formic acid and phosphate) in 125 samples of honey from different regions of Spain. The results were analyzed using a multivariate statistical study consisting of a principal component analysis and linear discriminant analysis resulting in a model that differentiates the honeys according to their geographical origin with a percentage of correct classification of 87.3%.

IDENTIFICATION OF BOTANICAL ORIGIN OF HONEYS BY THEIR PHYSICOCHEMICAL PROPERTIES AND MINERAL CONTENT

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The mineral content of honey is in a ratio less than 1%. It influences in honey such properties as colour, electrical conductivity as well as pH, and it is important because mineral content could give an indication of environmental pollution and herewith also an indication of the geographical and botanical origin of a given honey sample. In this study, colour parameters, electrical conductivity, pH and the mineral content of 125 honey samples from 12 different botanical origins (*Castanea sativa* Miller, *Citrus* sp., *Echium* sp., *Erica* sp., *Eucalyptus* sp., *Lavandula* sp., *Rosmarinus* sp., *Rosmarinus* and *Trifolium* sp., *Rubus* sp., *Trifolium* L. sp., multifloral, and blends of multifloral and honeydew honey) were studied. Results were submitted to unsupervised methods such as principal components analysis and supervised learning methods like linear discriminant analysis in order to evaluate possible data patterns, and the possibility of differentiating Spanish honeys according to their botanical origins. Principal components analysis explained 82.3% of the variance with the first six principal compontent variables. The linear discriminant analysis allowed correct botanical classification of 73.2% of the samples.

DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS OF HONEYS COMPARING FOURIER TRANSFORM INFRARED SPECTROSCOPY AND REFERENCE METHODS.

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Differentiation between nectar and honeydew honeys is based on sensory and microscopic characteristics and also includes physicochemical characteristics of the honeys, such as the electrical conductivity, the free acidity and the pH. Fourier Transform Infrared Spectroscopy (FT-IR) is faster and less laborious than the reference methods using a conductimeter, a pH meter or the reference method by equivalence point titration. Therefore we evaluated the suitability of the FT-IR method for the determination of some physicochemical measurands distinguishing floral and honeydew honeys.

Three different honey samples with low, intermediate and high electrical conductivities and free acidities were measured 10 times each at 10 different days using the FT-IR and the reference methods. The means and repeatability limits of the methods were compared. Additionally rape and

honeydew honeys with low and high electrical conductivities were included in the comparison.

The reference - and FT-IR methods for the pH and the water determination presented similar means and repeatability limits (r=2.83Sr) within the range that was tested, suggesting that both methods are equivalent.

The repeatability limits of the electrical conductivities and the free acidities determined by the FT-IR method were wider than those of the reference methods, but they were still in a range that is not critical for most analyses.

The means of the electrical conductivity of both methods were similar in the intermediate range of the calibration curve, suggesting that the FT-IR method is suitable for the discrimination of honeydew and floral honeys at 0.8mS/cm. However, the FT-IR method gave significantly higher values at the low range of the calibration curve. Therefore, the FT-IR method is not equivalent to the reference method in this range and not suitable for reliable discrimination of some monofloral honeys from polyfloral honeys at the low end of the calibration curve around 0.25-0.28 mS/cm. It may be necessary to recalibrate the FT-IR method separately for the low and the high range.

While the FT-IR methods for the pH and the water determination were equal to the reference methods, the conductimetric reference method for electrical conductivity was superior to the FT-IR method.

DETERMINATION OF BENZO(A)PYRENE IN BEESWAX .

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Polycyclic aromatic hydrocarbons (PAHs) are one of the most important groups of environmental pollutants, some of which are known or suspected carcinogens or mutagens to humans. They are widely present in the environment due to their lipophilic properties, which might allow their adsorption onto beeswax and the use of this bee product as a biological indicator.

Benzo(a)pyrene (BaP) is one of the most carcinogenic of the PAHs and it has been extensively studied, however, little information is available about this type of pollutants in beeswax and other bee products.

In this work, an analytical method for the identification and quantification of BaP in beeswax has been developed and evaluated. The method consists of a sample preparation with methanol, followed by a solid phase extraction (SPE) and a quantification performed using high-performance liquid chromatography (HPLC) with fluorescence detection. Analytical performance of the proposed method, including sensitivity, accuracy and precision was satisfactory.

Keywords: Benzo(a)pyrene; PAHs; beeswax; HPLC.
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IDENTIFICATION OF UNKNOWN COLOUR IN HONEY USING UPLC/TOF AND HPLC/MS/MS

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Customers in the Central Europe think that dark honey has better quality than light honey. Forest honey is usually darker than others. It is very easy to add unauthorized colour mixture to light honey or more often to honey diluted with inverted sugar syrups. Effect is that adulterated honey looks like forest honey. It is fraud, because according to the Council Directive 2001/110/EC is banned to add any food ingredients to honey. Unusual cheap pitch dark honey from market is therefore suspected.

This paper describes analytical strategy for identification this type of adulteration. At the very beginning of study was investigated what can be used as colorant. After asking from beekeepers we received to the laboratory unknown brown syrup. Next investigation confirmed that this unknown syrup is probably food additive E-150d using for colouring of beverages, breads etc. This syrup is mixture of several substances. We found then low amount of this syrup added to light honey has colorific effect. Therefore main goal of this study was to develop sensitive and selective analytical method applicable for identification of this syrup on lowest concentration in honey as soon as possible, the best around 50 $mg.kg^{-1}$. The first part of the analytical step was to identify major markers from this unknown solution. Diluted unknown brown syrup was infused using positive ESI to MicrOTOF Focus II, (Bruker Daltonic) TOF (Time of Flight) mass spectrometer. Average high resolution mass spectrum within range 100 – 1000 Da was acquired. After evaluation of data two candidate exact masses as possible markers were identified. Then separation conditions were developed using reverse phase liquid chromatography (UPLC) coupled with TOF. Extracted ion chromatograms showed that two selected exact masses are truly present in brown syrup but not in any type of pure honey. In general TOF is less sensitive than low resolution mass spectrometers worked under MS/MS conditions. Therefore was developed MS/MS fragmentation of one selected exact candidate mass using ion trap mass spectrometer (Gemini, Bruker Daltonic). Both analytical detection approaches showed good agreement but MS/MS is slightly more sensitive like TOF.
In last part of this study were analyzed 26 suspected forest honeys from market. In 11 honeys were detected markers comes from brown syrup what was surprised.

OCCURRENCE OF MICROSCOPIC ELEMENTS AND THEIR RELATION WITH THE ORIGIN OF HONEY.

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The microscopic analysis of honeys let to recognize different structures. Pollen grains have been the best studied elements, however others like spores of fungus or yeast are more unknown. Some of them come from primary sources and are associated at the presence of honeydew such *Alternaria*, *Stemphylium* or *Leptosphaeria*. In the case of yeast a variety occurs regularly in the floral nectar of many plant species, where they frequently reach extraordinarily high densities. This is the case of some species of *Metschnikowia* or *Candida*.
The aim of this work is to study some of these microscopic elements and their relation with the source of honey. The samples are from NW Spain and were provided for the beekeepers. We realize a physicochemical analysis includes: humidity, HMF, diastase and invertase content, sugars pattern, mineral content, pH, electrical conductivity, phenols content, flavonoids content and percentage of RSA; a melissopalynological analysis and a sensorial study.
It has been analyzed the relations between microscopic elements traditionally considered as honeydew elements, yeast and algae and physicochemical, sensorial and palynological parameters used for honey typification. The results have allowed to establish relationships on the honeydew contribution to the honeys and their botanical origin.

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COMPARISON OF AROMA PROFILE OF TURKISH AND GREEK PINE HONEY SAMPLES

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Pine honey is a unique type of honeydew honey, produced mainly in Aegean side of Turkey and Greece. It is possible to differentiate pine honey from other types of honey by its specific chemical, physical and sensorial characteristics. But further investigation is needed to determine the geographical origin of pine honey.

In this study, aroma compounds of a total of 30 pine honey samples from Turkey and Greece were analyzed by purge and trap, gas chromatography mass spectrometer system and the results were compared as an indicator of their geographical origin. For this purpose, 15 pine honey samples from 6 different regions of Turkey (Bergama, Marmaris, Köyceğiz, Söke, Muğla, Isparta) and 15 pine honey samples from 3 different regions of Greece (Evoia, Chalkidiki, Thassos) were analyzed. A total of 73 aroma compounds were isolated from Turkish samples and 89 compounds from Greek samples. Each sample showed a different aroma profile, with some compounds missing in some samples. 39 compounds were found to be present in all samples from Greece whereas only 10 compounds were common in samples from Turkey. 29 compounds were isolated from both Turkish and Greek pine honey samples.

18 compounds (2,5-dimethyl-furan, 2-methyl-3-buten-1-ol, 1-pentanol, methyl-benzene , 2-methyl-2-buten-1-ol, 3-methyl-2-butenal, 2-furan-carboxaldehyde , 3,3,5-trimethyl-1-cyclohexene, 1,4-dimethyl-benzene {p-xylene}, hexanol, 1-heptanal, 1-(2-furanyl) – ethanone, 6-methyl-5-heptan-2-one, 2-pentyl-furan, 1-methyl-4-(1-methyl-ethyl)-benzene , phenyl-acetaldehyde, cycloheptanomethanol, decanal) were found to be present only in Greek samples and *2-butanone* was specific to only Turkish samples. As a result, Turkish pine honey samples showed a higher variety depending on different production regions but still showing similar aroma characteristics which differentiates them from Greek pine honey samples. Also some compounds were considered as good markers both for Greek and Turkish pine honeys. It was concluded that aroma profiling is a useful, promising tool for determination of geographical origin which needs further investigation.